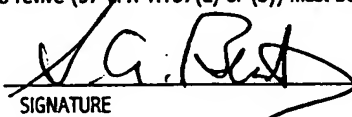


<b>TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371</b>		ATTORNEY'S DOCKET NUMBER  <b>081356-0239</b>
INTERNATIONAL APPLICATION NO. PCT/IP2003/012734	INTERNATIONAL FILING DATE 10/03/2003	U.S. APPLICATION NO. (If known see 37 C.F.R. 1.52) Unassigned <b>10/530207</b>
TITLE OF INVENTION HUMAN ARTIFICIAL CHROMOSOME (HAC) VECTOR		PRIORITY DATE CLAIMED 10/04/2002
APPLICANT(S) FOR DO/EO/US Mitsuo OSHIMURA, Motonobu KATOH, Kazuma TOMIZUKA, Yoshimi KUROIWA & Minoru KAKEDA Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.		
2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.		
3. <input type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.		
4. <input type="checkbox"/> The US has been elected (Article 31).		
5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))		
<input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau).		
<input checked="" type="checkbox"/> has been communicated by the International Bureau.		
<input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US)		
6. <input checked="" type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).		
<input checked="" type="checkbox"/> is attached hereto.		
<input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4).		
7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))		
<input type="checkbox"/> are attached hereto (required only if not transmitted by the International Bureau).		
<input type="checkbox"/> have been communicated by the International Bureau.		
<input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.		
<input checked="" type="checkbox"/> have not been made and will not be made.		
8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).		
9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).		
10. <input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).		
Items 11 to 20 below concern other document(s) or information included:		
11. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.		
12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.		
13. <input checked="" type="checkbox"/> A preliminary amendment.		
14. <input checked="" type="checkbox"/> An Application Data Sheet under 37 CFR 1.76.		
15. <input type="checkbox"/> A substitute specification.		
16. <input type="checkbox"/> A power of attorney and/or change of address letter.		
17. <input checked="" type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 37 CFR 1.821 - 1.825		
18. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4).		
19. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).		
20. <input checked="" type="checkbox"/> Other items or information: Microorganism Deposit receipt, Translation of Receipt, Declaration, Petition to Accept Color Photographs, PCT/IB/308; PCT/IB/304		

JCO6 Rec'd/PCT/PTO 04 APR 2005

U.S. APPLICATION NO. (If known, see 37 CFR 1.51) Unassigned <b>7530207</b>		INTERNATIONAL APPLICATION NO. PCT/JP2003/012734		ATTORNEY'S DOCKET NUMBER 081356-0239	
The following fees have been submitted:					
21.	<input checked="" type="checkbox"/> Basic national fee	\$300		\$	300.00
22.	<input checked="" type="checkbox"/> Examination fee			\$	200.00
If International preliminary examination report prepared by USPTO and all claims satisfy provisions of PCT Article 33(1)-(4)		\$100			
All other situations		\$200			
23.	<input checked="" type="checkbox"/> Search fee				
Search fee (37 CFR 1.445(a)(2)) has been paid on the international application to the USPTO as an International Searching Authority		\$100			
International Search Report prepared and provided to the Office		\$400			
All other situations		\$500		\$	500.00
TOTAL OF ABOVE 21, 22 and 23 =				\$	1000.00
<input checked="" type="checkbox"/> Additional fee for specification and drawings filed in paper over 100 sheets (excluding sequence listing or computer program listing filed in an electronic medium). The fee is \$ for each additional 50 sheets of paper or fraction thereof.					
Total Sheets	Extra sheets	Number of each additional 50 or fraction thereof (round up to a whole number)	RATE		
206 - 100 =	106 /50 =	3	x \$250.00	\$	750.00
Surcharge of \$130.00 for furnishing the oath or declaration later than 30 months from the earliest claimed priority date (37 CFR 1.492(e))				\$	130.00
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$	
Total Claims	66 - 20 =	46	x \$ 50.00	\$	2300.00
Independent Claims	5 - 3 =	2	x \$ 200.00	\$	400.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$ 360.00	\$	360.00
TOTAL OF ABOVE CALCULATIONS =				\$	4940.00
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. Fees above are reduced by 1/2.				+	\$ 0.00
SUBTOTAL =				\$	4940.00
Processing fee of 130.00 for furnishing the English translation later than 30 months from the earliest claimed priority date (37 CFR 1.492(i)).				\$	
TOTAL NATIONAL FEE =				\$	4940.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). 40.00 per property				\$	
TOTAL FEES ENCLOSED =				\$	4940.00
				Amount to be refunded:	
				charged:	
<p>a. <input checked="" type="checkbox"/> A check in the amount of \$4940.00 to cover the above fees is enclosed.</p> <p>b. <input type="checkbox"/> Please charge my Deposit Account No. <u>19-0741</u> in the amount of \$4940.00 to cover the above fees. A duplicate copy of this sheet is enclosed.</p> <p>c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>19-0741</u>. A duplicate copy of this sheet is enclosed.</p> <p>d. <input type="checkbox"/> Fees are to be charge to a credit card. WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.</p> <p>NOTE: Where an appropriate time limit under 37 CFR 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the International Application to pending status.</p>					
SEND ALL CORRESPONDENCE TO:					
Foley & Lardner LLP					
Customer Number: 22428					
 SIGNATURE Stephen A. Bent NAME 29,768 REGISTRATION NUMBER Date: April 4, 2005					

*IN THE UNITED STATES PATENT AND TRADEMARK OFFICE*

Applicant: Mitsuo OSHIMURA et al.  
Title: HUMAN ARTIFICIAL CHROMOSOME (HAC) VECTOR  
Int'l. Appl. No.: PCT/JP2003/012734  
Int'l. Filing Date: October 3, 2003  
Examiner: Unassigned  
Art Unit: Unassigned

PETITION TO ACCEPT COLOR PHOTOGRAPHS UNDER 37 CFR 1.84

Commissioner for Patents  
P.O. Box 1450  
Alexandria, Virginia 22313-1450

Sir:

In accordance with 37 CFR 1.84(a)(2), Applicants petition for the acceptance of Figs. 3, 4a, 4b, 6, 7a, 7b, 9, 10, 13, 14, 15a, 15b, 17, 18a, 18b, 20a, 20b, 21, 22a and 22b(photographs) to be entered in the above-captioned application.

In support thereof, Applicants submit:

- I) A fee in the amount of \$130.00 as is set forth under 37 CFR 1.17(h);
- II) Three (3) sets of each figure set forth above;
- III) An amendment to the specification indicating that the application contains color photographs.

04/08/2005 SNAJARRD 00000019 10530207

09 FC:1464

130.00 OP

In the Specification:

On page 1, directly beneath the title, please insert the following:

--The application contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.--

REMARKS

Fig. 3 is a photograph showing the results of southern blot analysis indicating the deletion of the distal region of the long arm in the puromycin resistant DT40 clone, or the introduction of an artificial telomere sequence in a site-specific manner.

Figs. 4a and 4b are photographs showing the results of FISH analysis indicating the deletion of the distal region of the long arm in the puromycin resistant DT40 clone. Figure 4a shows a human chromosome 21 (arrow) retained in the DT40 cell, and Figure 4b shows a human chromosome 21 fragment (arrow) from which the long arm has been deleted.

Fig. 6 is photographs showing the results of southern blot analysis (A) and PCR analysis (B) used to screen the blasticidin resistant DT40 clone for homologous recombinants (clones in which the loxP sequence was introduced into human chromosome 21 in a site-specific manner).

Fig. 7a and 7b are photographs showing the results of FISH analysis indicating the retention of a human chromosome 21 (fragment) in the blasticidin resistant CHO-K1 clone. Figure 7a shows a full-length (entire) human chromosome 21 before telomere truncation and Figure 7b shows a human chromosome 21 fragment from which the distal region of the long arm has been deleted.

Fig. 9 is a fluorescence microscope photograph showing GFP expression in the G418 resistant CHO-K1 clone.

Fig. 10 is a photograph showing the results of southern blot analysis indicating the occurrence of site-specific recombination in a loxP sequence in the G418 resistant CHO-K1 clone.

Fig. 13 is a photograph showing the results of southern blot analysis indicating the deletion of the distal region of the short arm in the hygromycin resistant DT40 clone, or the introduction of an artificial telomere sequence in a site-specific manner.

Fig. 14 is a photograph showing the results of PCR analysis indicating the deletion of the distal region of the short arm in the hygromycin resistant DT40 clone, or the introduction of an artificial telomere sequence in a site-specific manner.

Figs. 15a and 15b are photographs showing the results of FISH analysis indicating the deletion of the distal region of the short arm in the hygromycin resistant DT40 clone. Figure 15a shows a human chromosome 21 (arrow) devoid of the long arm retained in the DT40 cell, and Figure 15b shows a human chromosome 21 fragment (arrow) from which the long and short arms have been deleted.

Fig. 17 is a photograph showing the results of FISH analysis indicating the retention of a human chromosome 21 fragment (arrow) in the blasticidin resistant HT1080 cell clone.

Figs. 18a and 18b are fluorescence (Figure 18a) and phase-contrast (Figure 18b) microscope photographs showing GFP expression in the G418 resistant HT1080 clone.

Figs. 20a and 20b are photographs showing the results of FISH analysis indicating the retention of a human chromosome 21 fragment in the drug resistant E14 cell clone. Figure 20a shows a chromosome fragment (arrow) devoid of the distal region of the long arm, and Figure 20b shows a chromosome fragment (arrow) from which the distal region of the short arm has been further deleted.

Fig. 21 is a photograph showing the results of FISH analysis indicating the retention of a human chromosome 21 fragment (arrow) in the drug resistant hiMSC cell clone.

Figs. 22a and 22b are fluorescence microscope photographs showing GFP expression (Figure 22a) and staining with anti-beta tubulin antibody (Figure 22b) when ES cells retaining HAC were induced to differentiate into nerve cells *in vitro*.

Respectfully submitted,

Date 4 April 2005

By S. A. Bent

FOLEY & LARDNER LLP  
Customer Number: 22428  
\*22428\*  
22428

PATENT TRADEMARK OFFICE  
Telephone: (202) 672-5404  
Facsimile: (202) 672-5399

Stephen A. Bent  
Attorney for Applicant  
Registration No. 29,768

*IN THE UNITED STATES PATENT AND TRADEMARK OFFICE*

Applicant: Mitsuo OSHIMURA et al.  
Title: HUMAN ARTIFICIAL CHROMOSOME (HAC) VECTOR  
  
Int'l. Appl. No.: PCT/JP2003/012734  
  
Int'l. Filing Date: October 3, 2003  
  
Examiner: Unassigned  
  
Art Unit: Unassigned

DECLARATION

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450


Sir:

I declare:

1. A deposit of the microorganism identified as SC20, of Accession number FERM BP-7583, was made at the International Patent Organism Depository, National Institute of Advanced Industrial Science and Technology, AIST Tsukuba Central 6, 1-1, Higashi 1-Chome, Tsukuba-shi, Ibaraki-ken 305-8566 Japan and accepted under the provisions of the Budapest Treaty for patent purposes.
2. The deposit having Accession number FERM BP-7583 was made on May 9, 2001.
3. All restrictions on the availability to the public of the culture deposited will be irrevocably removed upon the granting of a patent from the above-identified application.
4. The deposit will be replaced if viable samples cannot be dispensed by the depository.
5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or

imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued therefrom.

Signed this 4<sup>th</sup> day of April 2005,

A handwritten signature in black ink, appearing to read "S.A. Bent", is written over a horizontal line.

Name: Stephen A. Bent  
Reg. No. 29,768  
Applicants' Representative



## INTERNATIONAL FORM

## BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

## RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

issued pursuant to Rule 7.1 by the  
INTERNATIONAL DEPOSITARY AUTHORITY  
identified at the bottom of this page.

## TO DEPOSITOR:

Name: KIRIN BEER KABUSHIKI KAISHA  
Representative: Koichiro ARAMAKI  
Address: 10-1, Shinkawa 2-chome, Chou-ku, Tokyo, Japan

<b>I. IDENTIFICATION OF MICROORGANISM</b>	
Identification Reference Given by the Depositor: SC20	Accession Number: FERM BP-7583
<b>II. A SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC POSITION</b>	
The microorganism identified under I above was accompanied by a document stating the following item(s). <input type="checkbox"/> A Scientific Property <input type="checkbox"/> Taxonomic Position	
<b>III. RECEIPT AND ACCEPTANCE</b>	
This International Depositary Authority accepts the microorganism identified under I above, which was received on May 9, 2001. (date of the original deposit)	
<b>IV. RECEIPT OF REQUEST FOR TRANSFER</b>	
This International Depositary Authority received the microorganism under I above on (date of the original deposit), and received on a request for transfer from the original deposit to the deposit under the Budapest treaty.	
<b>V. INTERNATIONAL DEPOSITARY AUTHORITY</b>	
Name: International Patent Organism Depositary National Institute of Advanced Industrial Science and Technology  Representative: <u>Dr. Yasuhiko KOMATSU, Director</u> (sealed)  Address: AIST Tsukuba Central 6, 1-1, Higashi 1-Chome, Tsukuba-shi, Ibaraki-ken 305-8566 Japan  Date: May 9, 2001	